Application Serial No.: 10/546,139 Inventor(s): Chateau et al.

Attorney Docket No.: 2912956-026000

AMENDMENTS TO THE CLAIMS

Claims 1 to 12. (Cancelled)

Claim 13. (Currently Amended) A method for producing an evolved protein involved in methionine biosynthesis pathway, the method comprising:

- a) generating a directed genetic modification in a gene of interest disrupting metE gene in an initial microorganism to yield a modified microorganism, wherein the production or consumption of a substrate is inhibited when the modified microorganism is grown on a defined medium, wherein the ability of the modified microorganism to grow is impaired when the modified microorganism is grown on a minimal medium containing no methionine. Sadenosylmethionine, homocysteine, or cystathionine;
- b) culturing the modified microorganism obtained in step (a) on the defined minimal medium for multiple generations, under selection pressure in the presence of methylmercaptan. allowing the modified microorganism to evolve a metabolic pathway to compensate for the impaired growth, wherein the defined medium can contain a co substrate promoting the evolution:
- c) selecting an evolved microorganism from step (b) able to grow on the defined minimal medium further comprising methylmercaptan, wherein at least one protein has evolved in the metabolic methionine biosynthesis pathway allowing the modified microorganism to produce methionine and proliferate; and
 - d) isolating the evolved protein.

Claim 14. (Currently Amended) The method as claimed in Claim 13, characterized in that wherein the produced isolated evolved protein is purified.

Claims 15 to 43. (Cancelled)

Claim 44. (Currently Amended) The method of claim 13, wherein disruption of the metE gene is performed by genetic modification comprises the directed mutation or deletion of a gene of interest the metE gene or the directed modification of a promoter of the metE gene in the gene of interest.

2

W 160606v1

Application Serial No.: 10/546,139 Inventor(s): Chateau et al. Attorney Docket No.: 2912956-026000

Claim 45. (Currently Amended) The method of claim 13, wherein the disruption genetic modification consists in the comprises removal of most of the metE gene of interest.

Claim 46. (Currently Amended) The method of claim 13, wherein the metE gene of interest is replaced with a selection marker gene.

Claim 47. (Currently Amended) The method of claim 13, wherein the microorganism is a bacterium selected among bacteria, yeasts and fungi.

Claim 48. (Currently Amended) The method of claim 13, wherein the microorganism is selected from Aspergillus sp., Bacillus sp., Brevibacterium sp., Clostridium sp., Corynebacterium sp., Escherichia sp., Gluconobacter sp., Pseudomonas sp., Rhodococcus sp., Saccharomyces sp., Streptomyces sp., Xanthomonas sp., or Candida sp.

Claim 49. (Currently Amended) The method of claim 43 48, wherein the microorganism is E. coli or C. glutamicum.

W 160606v1 3